



Strategies of the control of an outbreak of leptospiral infection in dairy cattle in Northeastern Brazil

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Abstract

The aim of the present study was to describe the strategies of the control of an outbreak of leptospiral infection in dairy cattle in Maranhão State, Northeastern Brazil. In the period from January to July 2015, 18 (17%) out of 106 cows presented abortion, six (5.7%) stillbirth, and 12 (11.3%) repeated estrus, totaling 24 animals with reproductive problems. The diagnosis of leptospirosis was based on serology (microscopic agglutination test—MAT), bacteriological culture, and polymerase chain reaction (PCR). Antibiotic therapy, vaccination protocols, and changes in management practices were suggested as control measures. Of all animals on the farm ($n = 280$), 136 (48.6%) were seropositive for at least one serovar of *Leptospira* sp. No pure leptospiral culture was obtained. Eight of the animals with reproductive problems yielded positive PCR results (vaginal fluid of seven animals and urine and vaginal fluid of one animal). Genetic sequencing of a vaginal fluid/urine PCR-positive sample revealed *Leptospira borgpetersenii*. One year after the adoption of control measures, no reproductive problems were observed. Thus, leptospirosis probably caused the reproductive failures in the herd, and the control and prevention measures implemented were efficient in controlling the disease.

Keywords *Leptospira* sp. · Control · Reproductive failures · Outbreak

Leptospirosis is a zoonotic disease of global importance, caused by pathogenic bacteria belonging to the genus *Leptospira*. The infection has a wide geographical distribution, with higher occurrence in tropical regions, and each serovar is usually associated with a maintenance host. Leptospirosis is important for cattle due to the compromised reproductive performance of the affected herds (Bourhy et al. 2014). In Brazil, investigations

of leptospirosis outbreaks in cattle have been reported (Mineiro et al. 2014). In Italy, two outbreaks of reproductive problems caused by *Leptospira borgpetersenii* serovar Hardjo in cattle have been reported (Mughini-Gras et al. 2014).

Cattle become infected mainly with the serogroup Sejroe (Pinto et al. 2016). However, any serovar can infect any animal species, but a limited number of serovars affect livestock species accidentally, leading to outbreaks of abortion, dead fetuses, and repeated estrus. Hardjo and Wolffi serovars are the most prevalently reported in studies on cattle in Brazil (Pimenta et al. 2014).

In outbreak situations, the control strategies used are measures of biosafety, vaccination, and selective chemoprophylaxis. Some improved measures have also been implemented, including pest control, extra environmental sanitation programmers, removal of piles of discarded material, closed herd maintenance, limiting access to contaminated water, banning intercropping, and supplying vitamins and mineral supplements (Mughini-Gras et al. 2014).

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Thus, the objective of the present study was to describe the strategies of the control of an outbreak of leptospiral infection in dairy cattle in Maranhão State, Northeastern Brazil.

The outbreak occurred from January to June 2015 in a dairy farm in the municipality of Timon, state of Maranhão, Northeastern Brazil. The region has a high annual rainfall (1383 mm). The herd was composed of 106 pregnant cows, 90 heifers, eight bulls, and 76 calves, totaling 280 animals. The heifers had not yet been covered, and covered cows that did not return to estrus were considered pregnant. The owner reported that 18 cows (17%) aborted in the last trimester of gestation, six (5.7%) had stillbirths, and 12 (11.3%) repeated estrus, totaling 24 animals with reproductive problems. The herd had never been vaccinated for leptospirosis. No clinical signs other than the reproductive problems were observed. The management system adopted at the farm was semi-intensive; rodent control was performed in the milking and feed storage facilities.

Blood samples were taken from all animals in the farm ($n = 280$) in July 2015, 15 days after reproductive problems were noted. Thirty days after this first visit, a new blood collection was performed, and urine and vaginal fluid samples were collected only from the animals that aborted, had stillbirths, or had repeated estrus ($n = 24$). Blood samples were collected by jugular venipuncture into 10-mL evacuated tubes, followed by serum extraction by centrifugation and storage at $-20\text{ }^{\circ}\text{C}$ until serology was performed. Urine was collected using a diuretic (furosemide, 2.5 mL/animal, intramuscularly), and vaginal fluid was collected with sterile swabs directly from the cervical region of the vagina and then stored in sterile 15-mL Falcon tubes with 2 mL of phosphate-buffered saline.

The serological diagnosis of leptospirosis was performed using the microscopic agglutination test (MAT) (OIE 2014). For leptospire isolation, immediately after, collection 1 mL of urine and vaginal fluid diluted in phosphate-buffered saline was seeded at the final concentration of 10% in semi-solid EMJH medium (Difco, BD Franklin Lakes, NJ, USA) with amphotericin B, 5-fluorouracil (1 mg/mL), fosfomycin (4 mg/mL), trimethoprim (0.2 mg/mL), and sulfamethoxazole (0.4 mg/mL) for inhibition of the proliferation of contaminating microorganisms (Chakraborty et al. 2011). After 24 h, 1 mL was seeded in EMJH medium with only 5-fluorouracil (1 mg/mL) added at the proportion of 10%, with subsequent incubation at $30\text{ }^{\circ}\text{C}$. The tubes were examined weekly for 6 weeks using dark-field microscopy.

DNA from urine and vaginal fluid was extracted using the kit Wizard Genomic SV DNA Purification System (Promega, Madison, USA). PCR and sequencing reactions were performed with the primers corresponding to nucleotides $38 \pm 57\text{ }5'\text{GGCGGCGCGTCTTAAACATG3}'$ and $348 \pm 368\text{ }5'\text{TCCCCCATTGAGCAAGATT3}'$ (Heinemann et al. 2000). The nucleotide sequence alignment was performed in

Seaview4. The sequence was aligned with reference *Leptospira* strains obtained from GenBank (National Center for Biotechnology Information, Bethesda, MD, USA) (<http://www.ncbi.nlm.nih.gov>), using the BLAST tool <http://www.ncbi.nlm.nih.gov/BLAST/>. A phylogenetic tree was generated using the software Seaview4. Phylogenetic trees were constructed based on the maximum-likelihood (ML) method with 1000 bootstraps, model TN 93, using PhyML 3.1. Trees were visualized in FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/>). The phylogenetic reconstruction program included sequences from *Leptospira* sp. for comparison (Fig. 1).

Seropositive animals were treated 10 days after the second visit with a single dose (25 mg/kg) of dihydrostreptomycin (Ourofino, Cravinhos, São Paulo, Brazil), and the seronegative animals were vaccinated with the commercial inactivated vaccine CattleMaster® 4 + L5 (Pfizer, Itapevi, São Paulo, Brazil), boosted after 30 days. Simultaneously, environmental measures were implemented, such as preventing the animals from accessing flooded areas and intensifying chemical and physical rodent control.

Of the 280 serum samples analyzed in the first visit, 136 (48.6%) were positive at MAT. The most frequent serogroups were Sejroe (92.7%), Tarassovi (5.1%), Hebdomadis (1.5%), and Australis (0.7%) (Table 1), with titers from 100 to 3200. Of the 24 cows with reproductive problems, eight (33%) were seropositive in the first visit, whereas 23 (95.8%) were seropositive in the second visit (Table 2), with titers from 100 to 400.

No pure leptospiral isolates were obtained; however, the DNA of pathogenic leptospire was detected in the vaginal fluid of eight animals, and in urine of one animal (Table 2). Of the eight PCR-positive samples, a nucleotide sequence obtained in one (295-bp fragment) showed 100% BLAST identity with *Leptospira borgpetersenii* sequences (Fig. 1). One year after the adoption of prevention and control measures, reproductive problems were no longer observed in the herd.

The serogroup Sejroe was the most frequent in this study, and it is reported as the most frequent in cattle. Recognized as being adapted to cattle, serovars of this serogroup are generally associated with several reproductive problems such as abortions, stillbirths, weak calves, and infertility. Thus, the reproductive problems observed may be related to the high frequency of these serovars in the herd (Tagliabue et al. 2016).

In conditions of high rainfall, as is the case in the studied region, excellent conditions are available for the survival and spread of leptospire (Robertson et al. 2012). However, infection influenced by environmental factors is more relevant when serogroups not adapted to the species in question are involved (Ellis 2015). Taking into account that the most frequent serovars in this study are

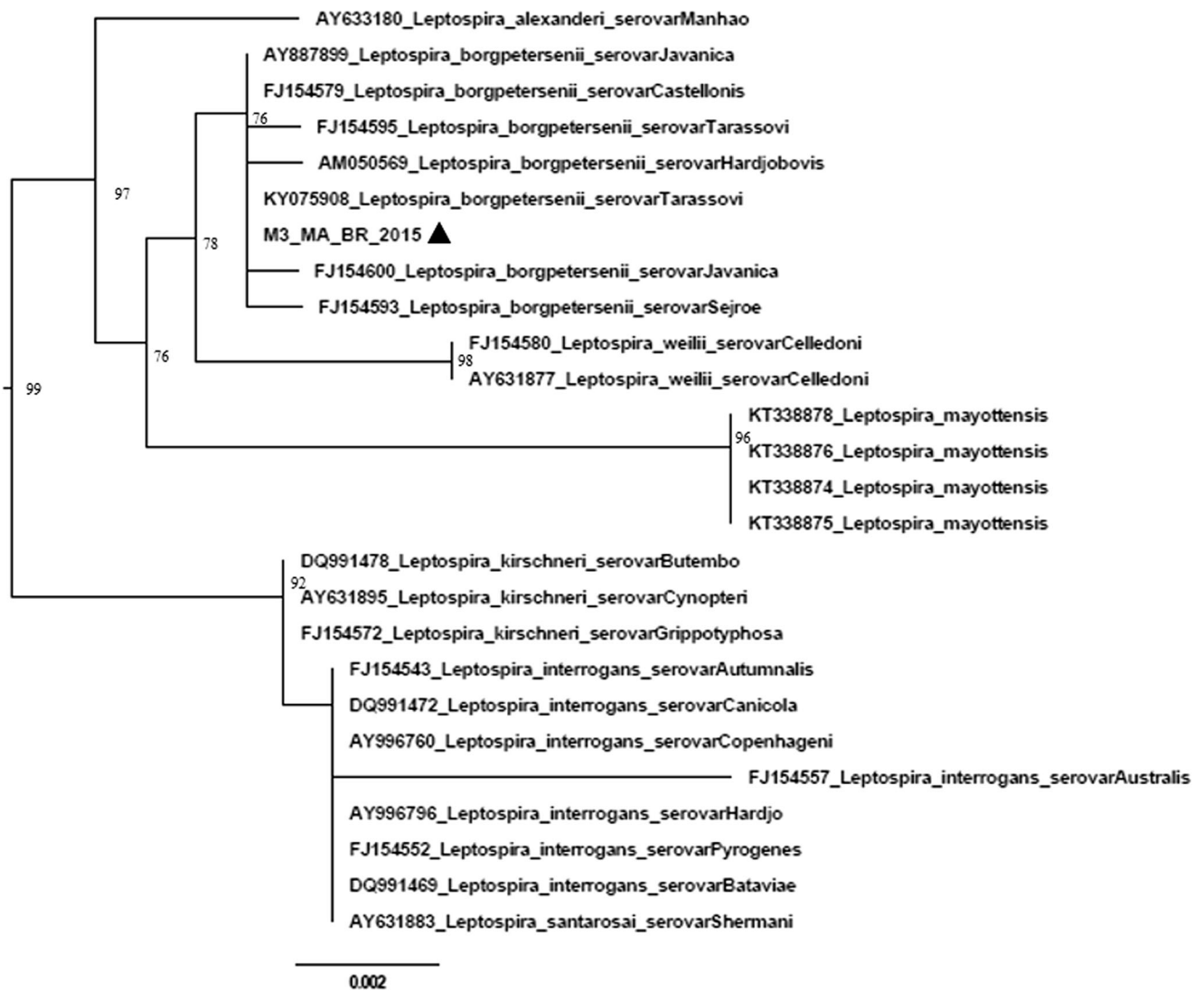


Fig. 1 The phylogenetic tree based on the fragment 16S rRNA gene sequences from *Leptospira* sp. was constructed based on maximum-likelihood phylogenetic tree model TN93. The analysis included 26 nucleotide sequences. Black triangle means sequenced sample

adapted to cattle (Wolffi, Hardjobovis, and Sejroe), bovine-to-bovine transmission was probably responsible for the outbreak, since in these cases, the elimination of

leptospire through urine is more constant, and the contact with the agent is facilitated (Correia et al. 2017).

Table 1 Most frequent *Leptospira* sp. serogroups in the first visit and respective titers in a leptospirosis outbreak in cattle in the state of Maranhão, Northeastern Brazil

Serogroup	Titers					Total	Frequency (%)
	100	200	400	800	3200		
Sejroe	45	58	19	3	1	126/136	92.7
Tarassovi	1	2	4			7/136	5.1
Hebdomadis	1	1				2/136	1.5
Australis	1					1/136	0.7
Total	48	71	23	3	1	136/136	100

A discrepancy was observed between the results of the two serologies in animals with reproductive problems, with eight animals positive (33%) in the first serology and 23 animals (95.8%) in the second. The non-detection of many positive animals in the first serology may be related to the short-lived immunity produced by infected animals, especially for serovars adapted to cattle (Adler 2015). However, as no control measure was performed in the period between the two collections, the animals may have been re-infected, thus explaining the detection of a higher proportion of positive animals in the second serology.

Host-adapted serovars, mainly serovar Hardjo, are related to reproductive disease in cattle, and in these cases, clinical signs such as abortion, return to estrus, and birth of weak

Table 2 Results of diagnostic tests in 24 cattle with reproductive problems in the second visit in a leptospirosis outbreak in the state of Maranhão, Northeastern Brazil

Animal	Reproductive failures	PCR/vaginal fluid	PCR/urine	1st serology/serovar/titer	2nd serology*/serovar/titer	Sequencing
M1	Abortion/repeated estrus	+	–	+/Wolffi/100	+/Tarassovi/400	–
M2	Abortion	+	–	+/Tarassovi/400	+/Tarassovi/Wolffi/400	–
M3	Abortion	+	+	–	+/Tarassovi/Hebdomadis/ Grippotyphosa/Wolffi/100	+
M4	Abortion	–	–	–	+/Tarassovi/200	–
M5	Abortion/repeated estrus	–	–	–	+/Tarassovi/100	–
M6	Abortion/repeated estrus	–	–	+/Wolffi/400	+/Wolffi/400	–
M7	Stillbirth	–	–	–	+/Tarassovi/100	–
M8	Abortion/repeated estrus	–	–	–	+/Tarassovi/100	–
M9	Abortion/repeated estrus	–	–	+/Hardjoprajitno/200	+/Grippotyphosa/400	–
M10	Abortion/repeated estrus	–	–	–	–	–
M11	Abortion	–	–	–	+/Tarassovi/200	–
M12	Abortion/repeated estrus	–	–	+/Sejroe/400	+/Hardjoprajitno/200	–
M13	Abortion/repeated estrus	–	–	–	+ / Tarassovi/100	–
M14	Abortion	–	–	–	+/Hebdomadis/Hardjoprajitno/200	–
M15	Stillbirth	–	–	–	+/Hebdomadis/Hardjoprajitno/200	–
M16	Stillbirth	–	–	–	+/Tarassovi/100	–
M17	Stillbirth	–	–	+/Wolffi/100	+/Tarassovi/200	–
M18	Abortion	–	–	–	+/Tarassovi/Wolffi/200	–
M19	Abortion	+	–	–	+/Tarassovi/400	–
M20	Abortion/repeated estrus	+	–	–	+ /Hardjoprajitno/200	–
M21	Abortion/repeated estrus	+	–	–	+/Wolffi/400	–
M22	Stillbirth	+	–	+/Wolffi/100	+/Grippotyphosa/200	–
M23	Stillbirth/repeated estrus	+	–	+/Wolffi/100	+/Wolffi/400	–
M24	Abortion/repeated estrus	–	–	–	+/Tarassovi/200	–

*30 days after first serology

animals are present (Mughini-Gras et al. 2014; Favero et al. 2017). In cattle, the DNA of *Leptospira* sp. has been identified in samples of vaginal fluid and in samples of cervicovaginal mucus and urine (Santana Oliveira et al. 2016). Positive PCR results from vaginal fluid samples suggesting the possibility of venereal transmission, although the pathogenesis of reproductive impairment in cattle is still not fully elucidated.

The acquisition of pure leptospiral cultures was not possible, and because the bacterium is very fastidious, isolation is not always a sensitive technique for the detection of leptospires, and failure is commonly reported. However, genetic sequencing revealed 100% identity with *L. borgpetersenii*, which belongs to the most frequently observed serogroup (Sejroe) in serology. The *L. borgpetersenii* serovar Hardjo strain Hardjobovis was isolated for the first time in cattle in Brazil and Latin America (Chideroli et al. 2016). In Italy, *L. borgpetersenii* serovar Hardjo strain Hardjobovis was also isolated from urine in two outbreaks of reproductive problems (abortions) in cattle (Mughini-Gras et al. 2014). Therefore, the identification of this species points to the importance of the

transmission of leptospires among cattle, acting as the main reservoirs for the bacterium within the herd.

Thus, based on the high frequency of seropositivity and carriers (PCR), leptospirosis can be inferred to be the cause of the reproductive problems, although no other collection of material for bacterial isolation, serology, or PCR was performed in the year after the adoption of control measures. The control of bovine leptospirosis carried out through an integrated program based on immunization, antibiotic therapy, and management changes has shown good results (Martins and Lilenbaum 2017). Vaccination is considered the cheapest method and essential measure for control. In cases of adapted strains, the associations of immunization with the treatment are efficient measures (Lilenbaum and Martins 2014). Changes in management also favor the control of the disease. Although serology and molecular analysis were not conducted 1 year after the application of the control measures, it is possible that a reduction of the disease occurred in the animals, because the sequels of the infection were reduced.

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Compliance with ethical standards

All procedures were conducted in accordance with Ethics Committee of the Animal Science Federal University of Campina Grande, Brazil.

Conflict of interest The authors declare that they have no conflicts of interest.

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